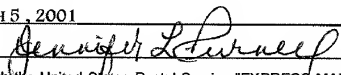| FORM PTO-1390 DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE (REV 1-98)	ATTORNEY'S DOCKET NO. 11362.0034.PCUS00; (INNS:034)
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371	U.S. APPLICATION NO. (If known, see 37 CFR 1.5) **09/786481**

INTERNATIONAL APPLICATION NO. **PCT/EP99/06592**	INTERNATIONAL FILING DATE **7 September 1999**	PRIORITY DATE CLAIMED **8 September 1998**

TITLE OF INVENTION
TAU AS A MARKER FOR EARLY CNS DAMAGE

APPLICANT(S) FOR DO/EO/US
Frank HULSTAERT; Eugeen VANMECHELEN; Hugo VANDERSTICHELE;

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.

2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. ☐ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2)).

    a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).

    b. ☒ has been transmitted by the International Bureau.

    c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).

6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).

    a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).

    b. ☐ have been transmitted by the International Bureau.

    c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.

    d. ☒ have not been made and will not be made.

8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 16 below concern document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. ☒ A FIRST preliminary amendment.
   ☐ A SECOND or SUBSEQUENT preliminary amendment.

14. ☐ A substitute specification.

15. ☒ A change of power of attorney and/or address letter.

16. ☒ Other items or information:

    Postcard, Fee Calculation Sheet (in duplicate);

H. 425544(94C_011.DOC)

U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 09/700481	INTERNATIONAL APPLICATION NO. PCT/EP99/06592	ATTORNEY'S DOCKET NUMBER 11362.0034.PCUS00; INNS:034

17. ☒ The following fees are submitted:

Basic National Fee (37 CFR 1.492(a)(1)-(5)):

	CALCULATIONS PTO USE ONLY

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $1,000.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $860.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO $760.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) $690.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) .... $ 100.00

ENTER APPROPRIATE BASIC FEE AMOUNT		=	$ 860.00	
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).			$ 0.00	

Claims	Number Filed	Number Extra	Rate		
Total Claims	15– 20 =		x $ 18.00	$	⎫
Independent Claims	4– 3	1	x $ 80.00	$ 80.00	⎬
Multiple dependent claim(s) (if applicable)				$ 270.00	⎭

TOTAL OF ABOVE CALCULATIONS		=	$	
Reduction by ½ for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (NOTE: 37 CFR 1.9, 1.27, 1.28)			$ .00	
SUBTOTAL		=	$ 1210.00	
Processing fee of $130.00 for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)).		+	$ .00	
TOTAL NATIONAL FEE		=	$ 1210.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property)		+	$ .00	
TOTAL FEES ENCLOSED		=	$	
			Amount to be refunded:	$ .00
			charged	$ .00

a. ☐ A check in the amount of $_____ to cover the above fees is enclosed. Applicants request a Notice of Missing Parts to satisfy the filing of the Declaration and Preliminary Amendment.

b. ☒ Please charge my Deposit Account No. 01-2508/11362.0034.PCUS00 in the amount of $1210.00 to cover the above fees. **A duplicate copy of this sheet is enclosed.**

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 01-2508/11362.0034.PCUS00. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Patricia A. Kammerer
HOWREY SIMON ARNOLD & WHITE, LLP
750 Bering Drive
Houston, TX 77057
(713) 787-1400

SIGNATURE

PATRICIA A. KAMMERER
NAME

29,775
REGISTRATION NUMBER

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

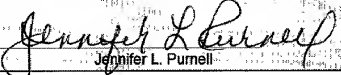|	
In re Application of:	§
FRANK HULSTAERT	§
EUGEEN VANMECHELEN	§ Group Art Unit: **Unknown**
HUGO VANDERSTICHELE	§
	§ Examiner: **Unknown**
Serial No.:	§
	§ Atty. Dkt. No.: **11362.0034.PCUS00**
Filed:	§ **INNS:034---**
	§
For: **TAU AS A MARKER FOR EARLY CNS**	§ History: **International Application No.:**
DAMAGE	§ **PCT/EP99/06592**
	§ **Filing Date: 7 September 1999**

PRELIMINARY AMENDMENT AND PRIORITY NOTICE

Box PCT
Commissioner for Patents
Washington, D.C. 20231

Sir:

Please amend this application as follows:

IN THE SPECIFICATION:

On page 1, line 2, please add a new paragraph following the Title:

--This application is a §371 national stage filing of PCT/EP99/06592, filed 7 September 1999 (published in English on 16 March 2000 as WO 00/14546) and claiming priority to EP 98870190.0 filed 8 September 1998.--

At page 4, line 1, please add a Summary Section:

--SUMMARY OF THE INVENTION

The present invention relates to a method for the early detection and/or quantification of CNS damage in an individual, said CNS damage being caused by space-occupying lesions of the CNS, by invasion of the CNS, by organisms, by anoxia or ischemia, by chemical agents, by physical agents or by a combination of these mechanisms. This method comprises the step of determining and/or quantifying the level of tau in an individual and comparing it to the level of tau in control healthy individuals.--

At page 5, line 27, please add a new Section:

--BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Tau values at diagnosis, before any treatment was given: 1. Control children; 2. AML; 3. AML-CNS+; 4. CML; 5. MDS; 6. B-NHL; 7. Non-B-ALL; 8. Non-B-ALL CNS+; 9. VHR non-B-ALL.

Figure 2. CSF-tau levels in seven patients after acture ischemic stroke. The CSF samples were collected at income (day 0-1), day 2-3, day 7-8, day 21-22 (3 weeks) and day 90-110 (3 months).

Figure 3. CSF-tau levels at the time of maximal release in relation to the size of the infraction as measured by CT scan.--

At page 20, please delete the section entitled "Figure Legends" on lines 5-15.

2

At page 26, line 13 the text should refer to table 4 instead of table 5, therefore please replace the paragraph on lines 11 - 16 to read:

--Tau levels in the CSF samples of patients with possible CNS damage caused by space occupying lesions, by invasion or metastasis, by bleeding, infarction or ischemia or by organisms and tau levels in CSF samples of control subjects are show in table 4. The group of patients that had possibly contracted CNS damage by space occupying lesions, by invasion or metastasis, by bleeding, by infarction or ischemia, or by organisms showed an overall higher tau level than the neurological control patients (p = 0.022, two sided Mann Whitney test).--

On a new page following the Claims Section, please add the Abstract found on the coversheet:

--ABSTRACT

The present invention provides a new method for the early diagnosis of CNS damage in an individual, said CNS damage being caused by space-occupying lesions of the CNS, by invasion or metastasis of the CNS, by organisms, by anoxia or ischemia, by chemical agents, by physical agents, or by a combination of these mechanisms. This new method comprises the step of determining and/or quantifying the level of tau in said individual and comparing it to the level of tau in control healthy individuals.--

IN THE FIGURES

Please replace original Fig. 2 with the attached corrected Fig. 2, where the legend has been changed from "Patent number" to read --Patient number--.

IN THE CLAIMS:

Please cancel claims 12 and 13.

Please amend claims 5 - 11, 15 and 16 as follows:

5. (Amended) A method according to [any of claims 1 to 4] <u>claims 1 or 2</u> in which the space-occupying lesion of the CNS is a primary brain tumor, benign or malignant, brain metastasis, or a subdural haematoma.

6. (Amended) A method according to [any of claims 1 to 4] <u>claims 1 or 2</u> in which the invasion or metastasis of the CNS is by leukemia, lymphoma or breast cancer.

7. (Amended) A method according to [any of claims 1 to 4] <u>claims 1 or 2</u> in which the organisms are bacteria or viruses causing encephalitis or meningitis.

8. (Amended) A method according to [any of claims 1 to 4] <u>claims 1 or 2</u> in which the anoxia or ischemia is caused by stroke, by cerebral infarction, by cerebral hemorrhage, by thrombosis, by perinatal asphyxia, by Binswanger disease or by vasculitis.

9. (Amended) A method according to [any of claims 1 to 4] <u>claims 1 or 2</u> in which the chemical agent is <u>gene therapy, pharmaceuticals, chemotherapy or exposure to chemical compounds</u>.

10. (Amended)   A method according to [any of claims 1 to 4] <u>claims 1 or 2</u> in which the physical agent is a trauma, stroke, intracranial pressure or radiation.

11. (Amended)   A method according to [any of claims 1 to 10] claims 1 or 2 in which CNS damage is detected and/or quantified in order to evaluate the effect of a certain treatment on said CNS damage.

15. (Amended)   A kit according to claim [14 for use in any method according to claims 1 to 11] 16, wherein the marker is tau.

16. (Amended)   A kit according to [claims 13 and/or] claim 14 characterised in that said kit comprises:

-     a monoclonal antibody (primary antibody) which forms an immunological complex with an epitope of tau;

-     a secondary antibody

    •   which can be a monoclonal antibody recognising an epitope of the tau-primary antibody complex, but not recognising the primary antibody alone, or

    •   which can be a polyclonal antibody recognising an epitope of the tau-primary antibody complex but not recognising the primary antibody alone, with said polyclonal antibody being preferably purified by immuno affinity chromatography using immobilized tau or immobilized tau-primary antibody complex;

-     a marker either for specific tagging or coupling with said secondary antibody,

- appropriate buffer solutions for carrying out the immunological reaction between the primary antibody and the test sample, between the secondary antibody and the tau-primary antibody complex and/or between the secondary antibody and the marker;

- [possibly,] optionally, for standardisation purposes, a purified protein or a synthetic peptide containing one or more tau epitopes.

REMARKS

The specification has been amended to claim priority and add reference to an earlier filed PCT application as required under 37 C.F.R. § 1.78(a)(2). Further, the specification has been amended to reflect the section headings and organization format preferred by the United States Patent and Trademark Office. Support for the Summary of the Invention is found at page 5, line 31 bridging page 6, line 5. Also attached is an Abstract on a new page.

In this amendment, claims 12 and 13 are cancelled and claims 5-11, 15 and 16 are amended to modify the recitation of multiple dependency. The amendment to claim 9 finds support in the Specification at page 7, lines 5 and 6. The amendment to claim 15 finds support in cancelled claim12. Claims 1-11 and 14-17 are now pending. A clean set of the pending claims is attached hereto.

It is believed that no fee is due. Should any fees under 37 C.F.R. §§ 1.16 to 1.21 be required for any reason relating to the enclosed materials, the Commissioner is authorized to deduct said fees from Deposit Account No. 01-2508/11362.0034.PCUS00.

CONCLUSION

In view of the foregoing amendments, applicants respectfully submit the claims are in proper form and condition for allowance. Applicants request that the claims be allowed and the application advanced to issue.

The Examiner is invited to contact the undersigned attorney at (713) 787-1438 with any questions, comments or suggestions relating to the referenced patent application.

Respectfully submitted,

Patricia A. Kammerer
Reg. No. 29,775
Attorney for Assignee
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HOWREY SIMON ARNOLD & WHITE, LLP
750 Bering Drive
Houston, Texas 77057-2198
(713) 787-1400

Date: March 5, 2001

PENDING CLAIMS FOLLOWING PRELIMINARY AMENDMENT

1.   A method for the early detection and/or quantification of CNS damage in an individual, said CNS damage being caused by space-occupying lesions of the CNS, by invasion or metastasis of the CNS, by organisms, by anoxia or ischemia, by chemical agents, by physical agents or by a combination of these mechanisms, said method comprising the step of determining the level of tau in said individual and comparing it to the level of tau in control healthy individuals.

2.   A method for the early in vitro detection and/or quantification of CNS damage in an individual, said CNS damage being caused by space-occupying lesions of the CNS, by invasion or metastasis of the CNS, by organisms, by anoxia or ischemia, by chemical agents, by physical agents or by a combination of these mechanisms, said method comprising the steps of:

-    obtaining a sample from said individual,

-    determining the level of tau in said sample and comparing it to the level of tau in control healthy individuals.

3.   A method according to claim 2 in which the sample is taken from the cerebrospinal fluid of the individual.

4.   A method according to claim 2 in which the sample is taken from the blood derivatives of the individual.

5. (Amended) A method according to claims 1 or 2 in which the space-occupying lesion of the CNS is a primary brain tumor, benign or malignant, brain metastasis, or a subdural haematoma.

6. (Amended) A method according to claims 1 or 2 in which the invasion or metastasis of the CNS is by leukemia, lymphoma or breast cancer.

7. (Amended) A method according to claims 1 or 2 in which the organisms are bacteria or viruses causing encephalitis or meningitis.

8. (Amended) A method according to claims 1 or 2 in which the anoxia or ischemia is caused by stroke, by cerebral infarction, by cerebral hemorrhage, by thrombosis, by perinatal asphyxia, by Binswanger disease or by vasculitis.

9. (Amended) A method according to claims 1 or 2 in which the chemical agent is gene therapy, pharmaceuticals, chemotherapy or exposure to chemical compounds.

10. (Amended)   A method according to claims 1 or 2 in which the physical agent is a trauma, stroke, intracranial pressure or radiation.

11. (Amended)   A method according to claims 1 or 2 in which CNS damage is detected and/or quantified in order to evaluate the effect of a certain treatment on said CNS damage.

14.   A kit for the early diagnosis of CNS damage in an individual, said CNS damage being caused by space-occupying lesions of the CNS, by invasion of the CNS, by organisms, by anoxia or ischemia, by chemical agents, by physical agents, or by a combination of these mechanisms, comprising a tool for the detection of tau.

15. (Amended)   A kit according to claim 16, wherein the marker is tau.

16. (Amended)   A kit according to claim 14 characterised in that said kit comprises:

-   a monoclonal antibody (primary antibody) which forms an immunological complex with an epitope of tau;

-   a secondary antibody

    •   which can be a monoclonal antibody recognising an epitope of the tau-primary antibody complex, but not recognising the primary antibody alone, or

    •   which can be a polyclonal antibody recognising an epitope of the tau-primary antibody complex but not recognising the primary antibody alone, with said polyclonal antibody being preferably purified by immuno affinity chromatography using immobilized tau or immobilized tau-primary antibody complex;

-   a marker either for specific tagging or coupling with said secondary antibody,

-   appropriate buffer solutions for carrying out the immunological reaction between the primary antibody and the test sample, between the secondary

antibody and the tau-primary antibody complex and/or between the secondary antibody and the marker;

- optionally, for standardisation purposes, a purified protein or a synthetic peptide containing one or more tau epitopes.

17. A method to screen or monitor the effect of compounds which prevent or treat CNS damage comprising the step of determining the level of tau and comparing it to the level of tau in a control sample.

ABSTRACT

The present invention provides a new method for the early diagnosis of CNS damage in an individual, said CNS damage being caused by space-occupying lesions of the CNS, by invasion or metastasis of the CNS, by organisms, by anoxia or ischemia, by chemical agents, by physical agents, or by a combination of these mechanisms. This new method comprises the step of determining and/or quantifying the level of tau in said individual and comparing it to the level of tau in control healthy individuals.

Figure 2

Figure 2

TAU AS A MARKER FOR EARLY CNS DAMAGE

FIELD OF THE INVENTION

5

The present invention relates to the field of CNS damage. The present invention relates to a new method for the early diagnosis of CNS damage by detection and/or quantification of tau.

10   ## BACKGROUND OF THE INVENTION

Damage of the central nervous system (CNS damage) is caused by various inducing agents among which different disease processes, physical or chemical agents, anoxia and ischemia. Disease processes include space occupying lesions, invasion or metastasis of the brain caused by different

15   malignancies and/or infection by a number of organisms.

Tumors of the CNS, may originate locally (primary tumors) or may spread to the CNS (metastases). Primary tumors arise from glial cells (astrocytoma, oligodendroglioma, glioblastoma), ependymal cells (ependymoma) or supporting tissue (meningioma, schwannoma, papilloma of the choroid plexus). In childhood, tumors arise from more primitive cells

20   (medulloblastoma, neuroblastoma, chordoma). Malignant astrocytoma or glioblastoma is the most common type of primary tumor in adults over age 20. Both benign and malignant primary CNS tumors are capable of producing neurologic impairment.

Leukemia is the most common type of cancer in children. During the last twenty years, the survival of children with leukemia has improved markedly based on the routine use of intensive

25   chemotherapy alone or as combined treatment (radiotherapy and chemotherapy). Currently, the estimated overall 10-year survival rate is around 75%. Given the increasing number of childhood leukemia survivors, concern has arisen about long-term effects of anti-cancer chemotherapy and/or radiotherapy resulting in possible damage to the central nervous system and the need for an early quantitative determination of this CNS damage is increasing.

30   Bacterial meningitis may be defined as an inflammation in response to bacterial infection of the pia-arachnoid and the fluid residing in the space which it encloses and also of the fluid in the

ventricles of the brain. The incidence of bacterial meningitis is between 4.6 and 10 cases per 100000 persons per year. *H. influenzae* is the most frequent cause, followed by *N. meningitidis* and *S. pneumoniae*. Once developed, characteristic features of bacterial meningitis include an increase in intracranial pressure, disruption of the blood-brain barrier, cerebral edema, and

5   alterations in cerebral blood flow. The longer the duration of meningitis and the less effective the treatment, the greater the chances that complications and neurologic residua will develop. Approximately 10 percent of infants and children who have bacterial meningitis will be left with persistent unilateral or bilateral sensory hearing loss. Approximately 30 percent of children who have had bacterial meningitis later will turn out to have subtle learning deficits (Wilson et al.,

10   1991).

Viruses can also affect the central nervous system in a variety of ways resulting in a distinction between viral meningitis, viral encephalitis, myelitis and CNS diseases due to slow virus infection. Other conditions that may cause CNS damage are chemical agents such as pharmaceuticals, chemotherapy or exposure to chemical compounds, and physical agents. Head injuries are

15   frequent in industrialised countries, affecting many patients in the prime of life. To appreciate the medical and social magnitude of this problem it needs only to be recognised that almost 10 million Americans have head injuries yearly, about 20 percent serious enough to cause brain damage. Another cause for CNS damage may be anoxia or ischemia. Anoxic-ischemic encephalopaty is a common and often disastrous condition, caused by a lack of oxygen to the brain, resulting from

20   hypotension or respiratory failure. Acute ischemic stroke causes neuronal damage and is a major cause of neurological handicap in western society. Perinatal asphyxia may be associated with CNS damage as well. To date, clinical, electroencephalographic and neuroradiologic evaluation, together with cerebral blood flow studies are the most readily available methods. However, early and accurate evaluation of the severity of brain damage after a hypoxic-ischemic event, remains

25   one of the most difficult problems in neonatal care.

For the detection of CNS invasion in leukemic children current diagnostic procedures include lumbar puncture, eye fundoscopy and brain imaging (Raichle, 1998). However, these diagnostic methods only allow detection of the CNS damage in a more advanced stage while already ongoing

30   CNS damage in the early stages may be missed by these methods. Therefore, there is a need for additional diagnostic methods that allow early detection of CNS damage.

3

A number of neurological markers have recently become available which reflect conditions of the central nervous system, relating to cell death, axon growth/re-induction, inflammation and/or blood-brain barrier dysfunction. The microtubule-associated protein tau exists in different

5   isoforms, of which 4 to 6 are found in adult brain but only 1 isoform is detected in fetal brain. The diversity of the isoforms is generated from a single gene on human chromosome 17 by alternative mRNA splicing (Himmler, 1989; Goedert et al., 1989; Andreadis et al., 1992). The most striking feature of tau protein, as deduced from molecular cloning, is a stretch of 31 or 32 amino acids, occurring in the carboxy-terminal part of the molecule, which can be repeated either 3 or 4 times.

10 Additional diversity is generated through 29 or 58 amino acid-long insertions in the NH_2-terminal part of tau molecules (Goedert et al., 1989). In vivo tau promotes microtubule assembly and stability in the axonal compartment of neurons by interactions involving its microtubule binding domain which is localised in the repeat region of tau (255-381) (Lewis et al., 1988). In normal circumstances adult brain contains 2 - 3 mol phosphate per mole of tau (Selden and Pollard, 1983;

15 Ksiezak-Reding et al., 1992). Phosphorylation of different sites in normal tau as studied in rat and humans is dependent on the developmental state (Lee et al., 1991; Bramblett et al., 1993; Goedert et al., 1993). Tau variants of 60, 64 and 68 kDa arising as a consequence of phosphorylation have been detected in brain areas showing neurofibrillary tangles (Delacourte et al., 1990; Goedert et al., 1992; Flament and Delacourte, 1990, Greenberg and Davies, 1990). These brains contain 6-8

20 mol phosphate per mol tau (Ksiezak-Reding et al., 1992). In tau isolated from paired helical filaments, phosphorylation can occur at several positions (Iqbal et al., 1989; Lee et al., 1991; Hasegawa et al., 1992). Detection of normally and abnormally phosphorylated tau in brain extracts is done either via antibodies (Mab Alz50: Ghanbari et al., 1990; Mab Ab423: Harrington et al., 1991; Mab AT120 : Vandermeeren et al., 1993; Mab AT180; Mab AT270 : International

25 application published under WO 95/17429 and Mab AT8 : International application published under WO 93/08302), or via the change in molecular weight (Flament and Delacourte, 1990), or else by functional assay (Bramblett et al., 1992). A combination of monoclonal antibodies, each recognising specific epitopes of tau, has been used to detect the presence of normally and abnormally phosphorylated tau in CSF (Van de Voorde et al., 1995). Tau has been used as a

30 marker to discriminate dementia with altered cytoskeletal properties such as Alzheimer's disease from normal aged subjects or from patients with other types of dementia.

AIMS OF THE INVENTION

It is an aim of the present invention to provide a method for the early detection and/or quantification of CNS damage in an individual, said CNS damage being caused by space-occupying lesions of the CNS, by invasion or metastasis of the CNS, by organisms, by anoxia or ischemia, by chemical agents, by physical agents, or by a combination of these mechanisms.

It is a more specific aim of the present invention to provide a method for the early detection and/or quantification of CNS damage in an individual, said CNS damage being caused a primary brain tumour, benign or malignant, brain metastasis or a subdural haematoma.

It is another more specific aim of the present invention to provide a method for the early detection and/or quantification of CNS damage in an individual, said CNS damage being caused by invasion of the CNS by leukemia, lymphoma or breast cancer.

It is another more specific aim of the present invention to provide a method for the early detection and/or quantification of CNS damage in an individual, said CNS damage being caused by bacteria or viruses causing encephalitis or meningitis.

It is another more specific aim of the present invention to provide a method for the early detection and/or quantification of CNS damage in an individual, said CNS damage being caused by stroke, by cerebral infarction, by cerebral haemorrhage, by thrombosis, by perinatal asphyxia, by Binswanger disease or by vasculitis.

It is another more specific aim of the present invention to provide a method for the early detection and/or quantification of CNS damage in an individual, said CNS damage being caused by chemotherapy.

It is another more specific aim of the present invention to provide a method for the early detection and/or quantification of CNS damage in an individual, said CNS damage being caused by trauma, stroke, intracranial pressure or radiation.

It is another aim of the present invention to provide a method for the early detection and/or quantification of CNS damage in an individual, said CNS damage being caused by space-occupying lesions of the CNS, by invasion or metastasis of the CNS, by organisms, by anoxia or ischemia, by chemical agents, by physical agents, or by a combination of these mechanisms in order to evaluate the effect of treatment on said CNS damage.

It is another aim of the present invention to provide a kit for the early diagnosis of CNS damage in an individual, said CNS damage being caused by space-occupying lesions of the CNS, by invasion of the CNS, by organisms, by anoxia or ischemia, by chemical agents, by physical agents, or by a combination of these mechanisms.

5   It is another more specific aim of the present invention to provide a kit for the early diagnosis of CNS damage in an individual, said CNS damage being caused a primary brain tumour, benign or malignant, brain metastasis or a subdural haematoma.

It is another more specific aim of the present invention to provide a kit for the early diagnosis of CNS damage in an individual, said CNS damage being caused by invasion of the CNS by

10   leukemia, lymphoma or breast cancer.

It is another more specific aim of the present invention to provide a kit for the early diagnosis of CNS damage in an individual, said CNS damage being caused by bacteria or viruses causing encephalitis or meningitis.

It is another more specific aim of the present invention to provide a kit for the early diagnosis of

15   CNS damage in an individual, said CNS damage being caused by stroke, by cerebral infarction, by cerebral haemorrhage, by thrombosis, by perinatal asphyxia, by Binswanger disease or by vascultitis.

It is another more specific aim of the present invention to provide a kit for the early diagnosis of CNS damage in an individual, said CNS damage being caused by chemotherapy.

20   It is another more specific aim of the present invention to provide a kit for the early diagnosis of CNS damage in an individual, said CNS damage being caused by trauma, stroke, intracranial pressure or radiation.

It is another aim of the present invention to provide a method to screen or monitor the effect of compounds which prevent or treat CNS damage.

25   All the aims of the present invention are considered to be met by the embodiments as set out below.

DETAILED DESCRIPTION OF THE INVENTION

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The present invention relates to a method for the early detection and/or quantification of CNS

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damage in an individual, said CNS damage being caused by space-occupying lesions of the CNS, by invasion of the CNS, by organisms, by anoxia or ischemia, by chemical agents, by physical agents or by a combination of these mechanisms. This method comprises the step of determining and/or quantifying the level of tau in an individual and comparing it to the level of tau in control

5   healthy individuals.

The present invention relates to the surprising finding that tau levels in CSF samples from children with leukemia are increased compared to upper limit values for healthy individuals. These increased tau levels are an indication of latent central nervous system invasion and already on-going CNS damage long before this CNS damage can be measured by the current diagnostic

10   procedures. Also in individuals that were suffering space occupying lesions of the CNS, invasion or metastasis of the CNS, ischemia, stroke or meningitis, increased tau levels were observed in an early stage. Accordingly, tau can be used as an aspecific marker for the early detection of CNS damage caused by invasion of the CNS by leukemia and in general, as an aspecific marker for the early detection of CNS damage caused by CNS damaging agents such as space-occupying lesion

15   of the CNS, invasion or metastasis of the CNS, organisms, anoxia or ischemia, chemical agents, physical agents, or a combination of these mechanisms.

The central nervous system (CNS) is that part of the nervous system which, in vertebrates, consists of the brain and spinal cord, to which sensory impulses are transmitted and from which motor impulses pass out, and which supervises and co-ordinates the activity of the entire nervous

20   system.

The term "CNS damage" refers to any condition of the CNS which is associated with a neuronal malfunctioning and which is caused by a specific inducing agent or damaging agent. More specifically, CNS damage refers to disease processes that include but are not limited to space-occupying lesions of the CNS, invasion or metastasis of the CNS and/or organisms. Space

25   occupying lesions may be, for example, primary brain tumours, benign or malignant, brain metastasis, parasite derived cysts such as *Taenia solium* or *Echinococcus granulosus*, hydrocephalus and/or subdural haematoma. Invasion or metastasis of the CNS can be caused by malignancies such as leukemia, lymphoma, breast cancer, lung cancer, melanoma and/or gastro-intestinal malignancies or other types of cancers. Organisms that may infect the CNS and cause

30   CNS damage include but are not limited to prions, viruses, bacteria or parasites. Bacteria and viruses that infect the CNS may cause meningitis, encephalitis, neuro aids or neuroborreliose.

They include but are not limited to *Neisseria meningitidis, H. influenzae, S. pneumoniae, Herpes simplex meningoencephalitis* and *Herpes simplex gluteolis.* CNS damage can also be caused by anoxia or ischemia during anestesia, perinatal asphyxia, drowning, asthma, stroke, cerebral infarction, thrombosis, cerebral haemorrhage, CO poisoning, Binswanger disease and/or vasculitis.

5   Chemical agents causing CNS damage include but are not limited to gene therapy, pharmaceuticals, chemotherapy and exposure to chemical compounds. CNS damage can also be caused by physical agents such as trauma, radiation, hypothermia, hyperthermia, intracranial pressure or stroke. It is also possible that more than one of the above mentioned causing agents are responsible for the CNS damage.

10   The present invention thus provides a method for the early detection and/or quantification of said CNS damage by determining the level of tau. "Early detection and/or quantification of CNS damage" means that the CNS damage is determined by a method that allows it to be detected before it is detectable by the current methods.

The term "tau" as referred to in the present application can be any form of tau, including any state

15   of phosphorylation. The level of tau is determined qualitatively and/or quantitatively as a measure for the degree of CNS damage. Tau can be detected in vitro as well as in vivo.

The method for the early in vitro detection of CNS damage in an individual comprises the steps of obtaining a sample from said individual, determining and/or quantifying the level of tau in said sample and comparing it to the level of tau in a sample of control healthy individuals. The term

20   "sample" refers to any source of biological material, for instance body fluids, hair, epithelial cells, peripheral blood or any other sample comprising tau protein. In a preferred embodiment, tau can be detected and/or quantified in vitro by analysis of the level of tau in a body fluid sample of the patient. The term "body fluid" refers to all fluids that are present in the human body including but not limited to blood, lymph, urine and cerebrospinal fluid (CSF). In a more specific embodiment

25   of the present invention tau is detected and/or quantified in a cerebrospinal fluid sample taken from the patient. In another specific embodiment of the invention tau is detected and/or quantified in a sample of blood derivatives of the patient. The blood sample can include the whole sample as taken from the patient. More preferably the blood sample includes a plasma sample or a serum sample.

30   Tau can be detected and/or quantified by any method known, including but not limited to the use of antibodies, the change in molecular weight (Flament and Delacourte, 1990), or else by

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functional assay (Bramblett et al., 1992). In a preferred embodiment tau can be detected by an
immunoassay comprising at least the following steps:

- obtaining a sample from the patient; and

- bringing said sample into contact with a monoclonal antibody (primary antibody or
5   capturing antibody) recognising tau, under conditions being suitable for producing an
antigen-antibody complex; and

- detecting the immunological binding of said antibody to said sample.

Advantageously, the monoclonal antibody used in the invention is in an immobilised state on a
suitable support. Alternatively, the present process may be put into practice by using any other
10   immunoassay format known to the person skilled in the art.

The process for the detection of the antigen can then be carried out by bringing together said
antigen-antibody complex formed by the antigen and the primary antibody recognising tau with:

a) a secondary antibody (or detector antibody)

*which can be a monoclonal antibody recognising an epitope of the tau-primary antibody
15   complex but not recognising the primary antibody alone, or

*which can be a polyclonal antibody recognising an epitope of the tau-primary antibody
complex but not recognising the primary antibody alone, with said polyclonal antibody
being preferably purified by immunoaffinity chromatography using immobilised tau or the
tau-primary antibody complex.

20   b) a marker either for specific tagging or coupling with said secondary antibody, with said
marker being any possible marker known to the person skilled in the art;

c) appropriate buffer solutions for carrying out the immunological reaction between the
primary antibody and the sample, between the secondary antibody and the tau-primary
antibody complex and/or between the bound secondary antibody and the marker, and,

25   d) possibly also, for standardisation purposes, a purified protein or synthetic peptide reactive
with the antibodies that recognise tau.

Advantageously, the secondary antibody itself carries a marker or a group for direct or indirect
coupling with a marker.

The term "epitope" refers to that portion of the antigen-antibody complex that is specifically
30   bound by an antibody-combining site. Epitopes may be determined by any of the techniques
known in the art or may be predicted by a variety of computer prediction models known in the

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art.

The expression "recognising", "reacting with", "immunological binding" or "producing an antigen-antibody complex" as used in the present invention is to be interpreted that binding between the antigen and antibody occurs under all conditions that respect the immunological

5   properties of the antibody and the antigen.

Any monoclonal or polyclonal antibody that specifically recognises tau may be used for the detection of tau. Antibodies specifically recognising normally and/or abnormally phosphorylated tau include Alz50 (Ghanbari et al., 1990), Ab423 (Harrington et al., 1991), AT8 (International application published under WO 93/08302), AT120 (Vandermeeren et al., 1993); AT180 and

10   AT270 (International application published under WO 95/17429) and AT100 (International application published under WO 96/04309). But also other antibodies known in the art that specifically recognise tau can be used.

The method for the early in vitro detection and/or quantification of CNS damage in an individual can also be used to evaluate the effect of a certain treatment on the CNS damage in said

15   individual. Possible treatments that might influence the status of the CNS include but are not limited to drug treatments, chemotherapy, physical therapy, including radiotherapy and gene therapy.

The method for the early in vivo detection and/or quantification of CNS damage in an individual comprises the steps of determining and/or quantifying the level of tau in said individual and

20   comparing it to the level of tau in control healthy individuals. In a preferred embodiment, tau can be detected in vivo by in vivo imaging. Tau can be visualised in situ by non-invasive methods including but not limited to brain imaging methods described by Arbit et al. (1995), Tamada et al. (1995), Wakabayashi et al. (1995), Huang et al. (1996), Sandrock et al. (1996), Mariani et al. (1997). These in vivo imaging methods may allow the localisation and visualisation of tau, for

25   example, by use of labelled antibodies recognising tau.

Tau can also be used as an aspecific marker for in vivo imaging to evaluate the effect of a certain treatment on the CNS damage in an individual. Possible treatments that might influence the status of the CNS include but are not limited to drug treatments, chemotherapy, physical therapy, including radiotherapy and gene therapy.

30   The present invention further relates to the use of tau as an aspecific marker for the manufacture of a diagnostic kit for the early detection in an individual of CNS damage caused by space-

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occupying lesions of the CNS, by invasion or metastasis of the CNS, by organisms, by anoxia or ischemia, by chemical agents, by physical agents, or by a combination of these mechanisms.

The present invention further relates to the use of tau as an aspecific marker for the manufacture of a diagnostic kit for the early detection in an individual of CNS damage caused by a primary

5   brain tumour, benign of malignant, brain metastasis, or a subdural haematoma.

The present invention further relates to the use of tau as an aspecific marker for the manufacture of a diagnostic kit for the early detection in an individual of CNS damage caused by invasion or metastasis of the CNS by leukemia, lymphoma or breast cancer.

The present invention further relates to the use of tau as an aspecific marker for the manufacture

10   of a diagnostic kit for the early detection in an individual of CNS damage caused by bacteria or viruses.

The present invention further relates to the use of tau as an aspecific marker for the manufacture of a diagnostic kit for the early detection in an individual of CNS damage caused by stroke, by cerebral infarction, by thrombosis, by cerebral haemorrhage, by perinatal asphyxia, by Binswanger

15   disease or by vasculitis.

The present invention further relates to the use of tau as an aspecific marker for the manufacture of a diagnostic kit for the early detection in an individual of CNS damage caused by chemotherapy.

The present invention further relates to the use of tau as an aspecific marker for the manufacture

20   of a diagnostic kit for the early detection in an individual of CNS damage caused by trauma, stroke, intracranial pressure or radiation.

The present invention further relates to a kit for the in vitro or in vivo diagnosis in an individual of CNS damage caused by space-occupying lesions of the CNS, by invasion or metastasis of the CNS, by organisms, by anoxia or ischemia, by chemical agents, by physical agents, or by a

25   combination of these mechanisms. Any kit that provides a tool for the detection of tau can be used for the diagnosis of the above-mentioned CNS damage.

A preferred kit for the in vitro diagnosis in an individual of CNS damage caused by space-occupying lesions of the CNS, by invasion or metastasis of the CNS, by organisms, by anoxia or ischemia, by chemical agents, by physical agents, or by a combination of these mechanisms is

30   based on an immunoassay and comprises:

- at least a monoclonal antibody (primary antibody) which forms an immunological

complex with an epitope of the tau protein;

- a secondary antibody

    * which can be a monoclonal antibody recognising an epitope of the tau-primary antibody complex but not recognising the primary antibody alone, or

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    * which can be a polyclonal antibody recognising an epitope of the tau-primary antibody complex but not recognising the primary antibody alone, with said polyclonal antibody being preferably purified by immunoaffinity chromatography using immobilised tau protein or immobilised tau-primary antibody complex;

10   - a marker either for specific tagging or coupling with said secondary antibody;

    - appropriate buffer solutions for carrying out the immunological reaction between the primary antibody and a test sample, between the secondary antibody and the tau-primary antibody complex, and/or between the bound secondary antibody and the marker;

15   - possibly, for standardisation purposes, a purified protein or synthetic peptide containing one of more tau epitopes.

. The present invention also relates to a method to screen or monitor the effect of compounds which prevent or treat CNS damage comprising the step of determining the level of tau and comparing it to the level of tau in a control sample.

20   The present invention will now be illustrated by reference to the following examples that set forth particularly advantageous embodiments. However, it should be noted that these examples are illustrative and can not be construed as to restrict the invention in any way.

Table 1: Treatment Protocol for patients with non-B-cell ALL/NHL (EORTC 58881).

Treatment phase	Drugs	Dose	Route	Days
Prephase	Prednisolone	60 mg/m2/d	PO	day 1 – 7
	MTX	according to age	IT	day 1 (*LP1*), day 8 (*LP2*), day 22 (*LP3*)
Protocol I: induction	Prednisolone	60 mg/m2/d	PO	day 8 – 28
(*37 days*)		20 mg/m2/d	PO	day 29 – 31
		10 mg/m2/d	PO	day 32 – 34
		5 g/m2/d	PO	day 35 – 37
	Vincristine	1.5 mg/m2	IV	day 8, 15, 22, 29
	Daunorubicine	30 mg/m2	IV	day 8, 15, 22, 29
	E coli asparaginase	10000 U/m2	IV	day 12, 15, 19, 22, 25, 29, 32, 35
Protocol I: consolidation	Cyclophosphamide	1000 mg/m2	IV	day 36, 63
(*26 days*)	6-mercaptopurine	60 mg/m2	PO	day 36 – 63
	Ara-C	75 mg/m2	IV	day 38 – 41, 45 - 48, 52 - 55, 59 – 62
(*14 days*)	MTX	according to age	IT	day 38 (*LP4*), 52 (*LP5*)

Table 1. Continued.

Treatment phase	Drugs	Dose	Route	Days
Interval therapy	6-mercaptopurine	25 mg/m2	PO	day 1 – 56
(56 days)	MTX	5000 mg/m2	IV	day 8, 22, 36, 50
	MTX	according to age	IT	day 9 (*LP6*), 23 (*LP7*), 37 (*LP8*), 51 (*LP9*)
(14 days)				
Protocol II: induction	Dexamethasone	6 mg/m2/d	PO	day 1 – 21
(35 days)	6-thioguanine	3 mg/m2/d	PO	day 22 – 35
		1 mg/m2/d	PO	day 26 – 29
	Vincristine	1.5 mg/m2	IV	day 8, 15, 22, 29
	Adriamycine	30 mg/m2	IV	day 8, 15, 22, 29
	E coli asparaginase	10000 U/m2	IV	day 8, 11, 15, 18
Protocol II: consolidation	Cyclophosphamide	1000 mg/m2	IV	day 36
(14 days)	6-thioguanine	60 mg/m2	PO	day 36 – 49
	Ara-C	75 mg/m2	IV	day 38 – 41, 45 – 48
	MTX	according to age	IT	day 38 (*LP10*)
(14 days)				
Maintenance	6-mercaptopurine	50 mg/m2/d	PO	Total duration of treatment = 2 years
	MTX	20 mg/m2 weekly	PO	

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Table 2. Treatment protocol for patients with B-cell NHL (UKCCSG 9602).

Treatment phase	Drugs	Dose	Route	Days
COP	Prednisolone	60 mg/m2/d	PO	day 1 – 7
(*7 Days*)	Vincristine	1 mg/m2	IV	day 1
	Cyclophosphamide	300 mg/m2	IV	day 1
	MTX	according to age	IT	day 1 (*LP1*)
	Hydrocortisone	according to age	IT	day 1 (*LP1*)
*** Start depending on the status of the patient				
COPADM 1 and COPADM 2	Prednisolone	60 mg/m2/d	PO	day 1 – 5
(*5 days*)	Vincristine	2 mg/m2	IV	day 1
	Cyclophosphamide	500 mg/m2/d	IV	day 2 – 4
	Doxorubicine	60 mg/m2	IV	day 2
	MTX	according to age	IT	day 2 (*LP2*), 6 (*LP3*)
		3000 mg/m2	IV	**day 1**
	Hydrocortisone	according to age	IT	day 2 (*LP2*), 6 (*LP3*)
*** Start depending on the status of the patient				

Table 2. Continued.

Treatment phase	Drugs	Dose	Route	Days
CYM 1 and CYM 2	MTX	according to age	IT	day 2 (*LP4*)
(*6 days*)		3000 mg/m2	IV	day 1
	Hydrocortisone	according to age	IT	day 2 (*LP4*), 7 (*LP5*)
	Ara-C	according to age	IT	day 7 (*LP5*)
		100 mg/m2/d	IV	day 2-6

*** Start depending on the status of the patient

COPADM 3	Prednisolone	60 mg/m2/d	PO	day 1 – 5
(5 days)	Vincristine	2 mg/m2	IV	day 1
	Cyclophosphamide	500 mg/m2/d	IV	day 2 – 3
	Doxorubicine	60 mg/m2	IV	day 2
	MTX	according to age	IT	day 2
		3000 mg/m2	IV	day 1
	Hydrocortisone	according to age	IT	day 2

IT = intra thecal; IV = intravenous, PO = per os, LP = lumbar puncture, MTX = methotrexate, Ara-C = arabinoside

Table 3: Treatment Protocol for patients with AML (EORTC 58921).

Treatment phase	Drugs	Dose	Route	Days
Induction	Ara-C	100 mg/m2	IV	day 1, 2
	Ara-C	200 mg/m2/d	IV	day 3 – 8
	Mitoxantrone	10 mg/m2/d	IV	day 3 – 5
	VP16	150 mg/m2/d	IV	day 6 – 8
	Ara-C	according to age	IT	day 1 (_LP1_), 8 (_LP2_)
First intensification	Ara-C	3000 mg/m2/d	IV	day 1 – 4
	Mitoxantrone	10 mg/m2/d	IV	day 5 – 7
Second intensification	Daunorubicin	20 mg/m2/d	IV	day 1 – 4
	Ara-C	200 mg/m2/d	IV	day 1 – 4
	VP16	100 mg/m2/d	IV	day 1 – 4
	6-thioguanine	100 mg/m2/d	PO	day 1 – 4
	Dexamethasone	6 mg/m2/d	PO	day 1 – 4
	Ara-C	according to age	IT	day 1 (_LP3_), 4 (_LP4_)
Third intensification	Ara-C	2000 mg/m2/d	IV	day 1 –3
	VP16	125 mg/m2/d	IV	day 2 – 5
Maintenance	6-thioguanine	40 mg/m2	PO	1 year
	Ara-C	40 mg/m2	SC	4 days/month

IT = intra thecal; IV = intravenous, PO = per os, SC = subcutanous, LP = lumbar puncture, Ara-C = arabinoside, VP16 =

Table 4. Level of tau in CSF samples of patients with possible CNS damage caused by different factors.

Cause of CNS damage[c]	Centre[a]	Patient	Age	Sex[b]	Tau level (pg/ml)
SPACE OCCUPYING LESIONS, INVASION OR METASTASIS					
Subdural haematome	01	025	39	M	263
Anaplastic oligoastrocytoma	01	032	68	M	329
Cerebral metastasis	08	024	70	F	292
Oligodendroglioma WHO III	08	031	48	M	335
Metastasis of breast cancer	08	020	52	F	150
BLEEDING, INFARCTION OR ISCHEMIA					
Stroke - CVA	01	021	62	F	451
Multiple lacunar stroke	01	026	56	F	250
Stroke - CVA	04	004	50	F	105
Acute but limited stroke	05	032	72	M	248
Subarachnoid hemorrhage	08	019	50	M	216
Chronic post–anoxia (vegetative state)	05	051	37	F	446
Binswanger disease	10	014	74	M	347
Vasculitis	10	009	57	F	1250
Cerebral ischemia	10	005	80	M	273
Ischemia	10	006	71	F	574
Superior sagittal sinus thrombosis	01	023	41	M	773

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Table 4. Continued.

Cause of CNS damage[c]	Centre[a]	Patient	Age	Sex[b]	Tau level (pg/ml)
ORGANISMS					
Cryptococcal meningitis	05	033	29	M	1143
Neuroborreliose	05	045	16	M	141
Chronic meningitis, unknown cause	05	046	34	F	452
Meningococcal meningitis	05	049	24	M	37
Bacterial meningitis	05	059	73	F	446
Neuro aids	05	060	45	F	237
Pneumococcal meningitis	05	063	70	M	111
Bacterial meningitis	06	026	25	M	1250
Bacterial meningitis	06	027	19	M	37
TBC meningitis	06	028	54	M	720
Bacterial meningitis	06	029	23	M	37
Bacterial meningitis	06	030	28	M	88
Viral meningitis	06	037	82	M	314
Bacterial meningitis	08	018	49	F	205
Viral meningitis	08	029	29	F	170
CONTROLS					
Muscle weakness and hysterical conversion	01	020	39	F	282
Facial palsy (peripheral nerve disease, normal CSF	05	041	44	M	141
Tension (psychogenic) headache	05	031	49	F	37
Psychogenic headache and neurosis	05	036	56	F	131
Psychogenic signs and symptoms	05	040	48	F	220
Major hysteria	05	042	47	F	471
Lumbar disc prolaps and sciatica	05	047	74	M	195

Table 4. Continued.

Cause of CNS damage[c]	Centre[a]	Patient	Age	Sex[b]	Tau level (pg/ml)
CONTROLS					
Control (associated Sjogren disease)	05	065	52	F	107
Control (neurosis)	05	079	40	F	90
Depression	10	008	59	F	201
Depression	10	010	69	F	139
Myalgia, myositis	04	003	31	M	37
Myalgia, myositis	04	005	35	F	435
Malaise, fatigue	04	006	50	M	98
Neck pain, vertigo	08	011	39	F	192
Headache	08	017	49	M	113
Depression	08	021	57	M	361
Headache, strabismus convergens	08	033	32	F	239
Bell's palsy	06	031	38	M	90
Bell's palsy	06	032	22	F	131
Bell's palsy	06	033	73	F	231
Bell's palsy	06	034	74	F	144
Carpal tunnel syndrome	06	035	71	M	190
Carpal tunnel syndrome	06	036	90	M	665

[a] 01: Prof. P. Cras, University Hospital, Neurology, Antwerp, Belgium and Dr. A. Daniels, UIA, Laboratory of Neurobiology, Antwerp, Belgium; 04: Dr. P.D. Mehta, Institute Basic Research, Staten Island, NY, USA; 05: Dr. A. Ivanoiu, UCL St-Luc, Laboratory of Neurochemistry, Brussels, Belgium; 06: Prof. P.P. De Deyn, UIA, Laboratory of Neurochemistry, Antwerp, Belgium; 08: Dr. C. Bancher, Lainz Hospital, Neurology, Vienna, Austria; 10: Dr. J. Wiltfang, Georg-August University, Gerontopsychiatry, Göttingen, Germany.

[b] F: female; M: male.

[c] In cases where multiple factors may contribute to the damage, only the factor supposed to be

most relevant is given.

5   FIGURE LEGENDS

Figure 1. Tau values at diagnosis, before any treatment was given: 1. Control children; 2. AML;
3. AML-CNS+; 4. CML; 5. MDS; 6. B-NHL; 7. Non-B-ALL; 8. Non-B-ALL CNS+; 9. VHR
non-B-ALL.

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Figure 2. CSF-tau levels in seven patients after acute ischemic stroke. The CSF samples were
collected at income (day 0-1), day 2-3, day 7-8, day 21-22 (3 weeks) and day 90-110 (3 months).

Figure 3. CSF-tau levels at the time of maximal release in relation to the size of the infarction as
15   measured by CT scan.

EXAMPLES

20   **Example 1: Increased tau levels in children with leukemia**

To evaluate the influence of chemotherapy on neuronal damage, a longitudinal study was
conducted, involving 65 children with leukemia (aged 2 to 16 years) without measurable central
nervous system involvement, and treated according to standard procedures. A total of 377 CSF
25   samples were analysed. Before each injection, a small volume of fluid was sampled for routine
laboratory analysis and the leftover was used in our study. These children were being diagnosed
and then treated for their leukemia at the University Hospital of Leuven, Belgium. Tau protein
in cerebrospinal fluid was assessed using the INNOTEST hTAU Antigen (Innogenetics, Gent,
Belgium).

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For all children suspected to have leukemia, a lumbar puncture was performed before the start of

the treatment to detect the possible presence of leukemic cells, indicative of central nervous system invasion. The tau levels measured at that time, and thus before treatment, would serve as the control level to compare chemotherapy-induced changes in the levels of tau.

5   We observed that some children with leukemia already had very high tau levels at diagnosis in spite of the fact that no leukemic cells were detected in the central nervous system. These children constitute a new risk group having brain invasion or leukemia-induced CNS damage, which cannot always be found using current diagnostic procedures (imaging of the brain, lumbar puncture, eye fundoscopy). This was further supported by the increased tau levels seen in one

10 patient having leukemia with proven cellular invasion into the brain (malignant cells in the cerebrospinal fluid).

Example 2: Increased tau levels in patients with leukemia before treatment

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1. Subjects

Between August 1996 and June 1999, 510 samples of CSF were taken from 82 children being treated for cancer at the Pediatric Hemato-oncology Department of the Catholic University of

20 Leuven, Belgium. CSF samples were only taken in the course of scheduled lumbar punctions (LPs) for staging or treatment for malignancy.

Three groups of patients with hematological malignancies were studied. The largest group consisted of 48 patients with non-B-ALL, treated according to the EORTC protocol 58881 (table 1). Of these children, 20 had CD10(+) blasts (or common ALL), from whom two patients had

25 also Down syndrome (DS) and 1 patient had the Brachmann-de Lange syndrome; 6 patients had common B-cell blasts, 2 patients had common T-cell blasts, 2 patients had pro-B-cell blasts, 9 patients had pre-B-cell blasts, and 9 patients had T-cell blasts. Fourty-two children had leukemia, 6 patients had non-Hodgkin's lymphoma stage II (1 patient), Stage III (4 patients) or Stage IV (1 patient). One patient had overt CNS involvement (CNS+), defined according to the study

30 protocol with malignant cells in the CSF. Five patients were considered as very high risk (VHR) patients according to the criteria defined in the protocol (2 patients with t(9;22) and 3 patients

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with corticoid-resistance). As the first part of the induction treatment was similar to the other patients, they were included in the analyses according to the induction chemotherapy. Twenty-eight of the patients within non-B-ALL could be followed longitudinally during their treatment period.

5   A second group of patients included 10 B-cell non-Hodgkin's lymphoma patients, treated according to the United Kingdom Children Cancers group (UKCCSG 9602) NHL protocol (table 2). Five patients had B-cell lymphomas, 3 patients had Burkitt's lymphoma, and 2 patients had anaplastic large cell lymphoma (ALCL). All patients were treated with the same protocol, except one patient who had B-cell leukemia. Six of these patients were studied longitudinally.

10   A third patient group consisted of 9 children with Acute Myeloid Leukemia - myelodysplasia (AML - MDS), of which 2 patients had CNS involvement and two patients had Down syndrome. There were 3 patients with M0, and 1 patient each with M1, M2, M5a or M7 phenotype. Two patients had MDS, of which one had developed AML and was treated with chemotherapy. All these patients, except one MDS patient, were treated according to the EORTC 58921 protocol

15   (table 3) and 7 patients were followed longitudinally.

The other patients consisted of a heterogeneous group of children (n = 9) in which for clinical reasons an LP was performed. This group includes 3 children with medulloblastoma (staging), 2 children with rhabdomyosarcoma (staging), 1 child with Langerhans cell histiocytosis (LCH, staging), ganglioglioma (staging), germinoma (staging), and retinoblastoma with CNS metastasis

20   (staging and follow up). The control group consisted of 4 children from whom CSF was taken as part of the routine control for possible viral or bacterial infections, but with negative results. One patient with a localized retinoblastoma (staging) and one screened for familial hemophagocytic lymphohistiocytosis (HLH) were also included in the control group. The nearest relatives of the patients gave oral informed consent for participation in the study.

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2. Methods

Liquor sampling. Lumbar punctures were performed just prior to the IT administration of therapeutics. Five ml of CSF was collected in different polypropylene tubes. One sample was

30   centrifuged immediately at 1500 rpm for 2 minutes to eliminate cells and other insoluble material. The supernatant was stored at −70°C for subsequent analysis. The number of freeze/thaw cycles

was restricted to a minimum.

Measurement of tau in the CSF. All biochemical analyses for the detection of CSF-tau were made without knowledge of the clinical diagnosis. Potentially confounding factors, such as the 5   number of freeze/thaw cycles, recipient type, and volume of sample per assay were standardized for the whole study protocol. CSF-tau levels were determined using a sandwich ELISA (INNOTEST hTAU Antigen, Innogenetics, Gent, Belgium), that measured total tau (both normal and hyperphosphorylated tau). Samples were analyzed firstly alone, at the time of each LP Afterwards, all samples derived from one patient were analyzed again on one immunoplate. The 10   correlation coefficient between the results from the first and the second approach for a set of 104 samples was 0.901 (95%CI: 0.856 - 0.933). The increases of CSF-tau described in the paper were excluded to be general increases of proteins in the liquor.

3. Results

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Normal upper limit levels for tau were firstly determined on CSF samples from the 6 control children. The mean CSF-tau value was 106.2 pg/ml (95%CI = 34.3-178.0). Arbitrary cut-off normal value was considered as 312 pg/ml (mean + 3 SD), which is in the range of values observed in adults (Hulstaert et al., 1999). Moreover, we did not find a correlation between the 20   CSF-tau values at diagnosis and the age of the children (Pearson $r = -0.161$, CI95% = -0.3914 - 0.08836, n = 64), suggesting that age has no influence on CSF-tau levels. Clearly pathological CSF-tau values can be considered as above 500 pg/ml.

Tau levels at diagnosis were analyzed for each subgroup of patients (Fig. 1). There was no 25   obvious difference for the tau levels in patients with and without Dow's syndrome (not shown). The two patients with overt CNS invasion (CNS+) had levels of CSF-tau at diagnosis above 312 pg/ml. However, the 2 children with MDS, 7/28 children with non-B-ALL, 1/4 non-B-ALL patients with very high risk criteria, 1/5 patients with AML, and 2/8 patients with B-cell NHL had a level of tau above 312 pg/ml, while using classical diagnostic procedures, CNS invasion was not 30   detected. Three patients with risen intracranial pressure (medulloblastoma), and one patient with germinoma, from which CSF was taken for staging, had high CSF-tau concentrations

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(823,1397,1500 and 442 pg/ml), in contrast to one patient with grade I astrocytoma who had a normal CSF-tau level (97 pg/ml). One patient with LCH had a normal CSF-tau level of 112 pg/ml. Two patients with rhabdomyosarcoma could be analyzed at diagnosis: one patient with stage I disease had a CSF-tau level of 279 pg/ml, the other patient with stage IV disease had a level of

5   320 pg/ml. One patient with retinoblastoma and CNS involvement had a CSF-tau level of 1800 pg/ml.

For 33 patients with non-B-ALL, CSF-tau levels at diagnosis did not correlate with tumor burden, as reflected by the white blood cell count (Pearson r = 0.04575, CD95%: -0.3024 - 0.3831) or serum LDH (Pearson r = -0.03002, CI95%: -0.3696 - 0.3166). In patients with B-NHL, there was

10   no significant correlation between the LDH level and CSF-tau (Pearson r = -0.3723, CD95%: -0.8532 - 0.4507).

Example 3: Use CSF-tau as a marker for early detection of possible CNS damage caused

15   **by stroke**

1. Subject

Seven patients, 3 men and 4 women, 63-81 years old (mean SD, 70.7±7.2 years) with cerebral

20   infarctions admitted to the Unit of Neurology, Sahlgren's University Hospital, Göteborg, Sweden, were incorporated in the study. All patients were included within 72 h of stroke onset.

2. Methods

25   CSF samples were collected using lumbar puncture. 12 ml was collected and frozen in 0.5 ml aliquots at -80°C until analyzed. CSF samples were collected at income (day 0-1), day 2-3, day 7-8, day 21-22 (3 weeks) and day 90-110 (3 months). CSF-tau levels were determined using a sandwich ELISA (INNOTEST hTAU Antigen, Innogenetics, Gent, Belgium), that measured total tau (both normal and hyperphosphorylated tau).

30   The extent of brain damage was examined with computerized tomography (CT) of the brain during the first day of admittance. Clinical the patients were also examined using the modified

Scandinavian Stroke Scale Index (SSI, Scandinavian Stroke Study Group, 1985) at onset of stroke and 3 months later for the degree of disability with the Bartel Index (BI; Mohoney and Barthel, 1965).

5   **3. Results**

CSF-tau showed a marked increase after acute stroke, with a peak after 1-3 weeks and return to normal after 3 months (Fig 2). There was also a correlation between CSF-tau levels and the size of the infarction as measured by CT scan (Fig 3). These results indicate that CSF-tau reflects

10   neuronal damage and degeneration and the level in the CSF depends on the amount of damaged nerve cells. In this study no correlation was found between the clinical data (SSI or BI) and CSF-tau, probably due to small number of patients.

15   **Example 4: Use of tau as a marker for early detection of possible CNS damage caused by different damaging agents**

1. Subjects

20   A multicentre study was carried out at 8 European and 2 U.S. university centres involved in CSF research, based on residual CSF archived at the centres for research purposes. CSF samples of patients with a broad range of different neurological conditions were included in order to get a general idea about the specificity of the tau marker changes in a variety of pathologies involving the CNS. Neurological controls consisted of subjects without obvious CNS damage (table 4).

25

The study was conducted in accordance with local clinical research regulations. If required additional local Ethics Committee or Institutional Review Board approval was obtained by the investigator prior to the start of the study.

30   **2. Methods**

CSF samples were collected using lumbar puncture (LP). Only CSF samples containing less than 500 red blood cells per μl were included in the study. The CSF samples were centrifuged at 2000 g for 10 minutes within 4 hours after LP and kept frozen without thawing. CSF samples from centre 01 had undergone an additional freeze-thaw cycle before analysis. The concentration of

5   total tau comprising normal tau and paired helical filament-tau was measured at the centres using a sandwich ELISA technique (INNOTEST hTAU-Antigen, Innogenetics N.V.).

3. CSF-tau levels in patients with possible CNS damage compared to CSF-tau levels in neurological control subjects

10

Tau levels in the CSF samples of patients with possible CNS damage caused by space occupying lesions, by invasion or metastasis, by bleeding, infarction or ischemia or by organisms and tau levels in CSF samples of control subjects are shown in table 5. The group of patients that had possibly contracted CNS damage by space occupying lesions, by invasion or metastasis, by

15   bleeding, by infarction or ischemia, or by organisms showed an overall higher tau level than the neurological control patients ($p = 0.022$, two sided Mann Whitney test).

REFERENCES

20

Andreadis A., Brown W., Kosik K. (1992) Structure and novel exons of the human tau gene. Biochem. 31: 10626-10633.

Arbit E., Cheung N.K., Yeh S.D., Daghighian F. ,Shang J.J., Cordon-Cardo C., Pentlow K.,
25   Canete A., Finn R., Larson S.M. (1995) Quantitative studies of monoclonal antibody targeting to disialogangliosid GD2 in human brain tumours. Eur. J. Nucl. Med. 22: 419-426.

Bramblett G., Trojanowski J., Lee V. (1992) Regions with abundant neurofibrillary pathology in human brain exhibit a selective reduction in levels of binding-competent tau and accumulation of
30   abnormal tau isoforms (A68 proteins). Lab. Invest. 66: 212-222.

27

Bramblett G., Goedert M., Jakes R., Merrick S., Trojanowski J., Lee V. (1993) The abnormal phosphorylation of tau at Ser396 in Alzheimer's disease recapitulates phosphorylation during development and contributes to reduced microtubule binding. Neuron. 10: 1089-1099.

5   Delacourte A., Flament S., Dibe E., Hublau P., Sablonniere B., Hemon B., Sherrer V., Defossez A. (1990) Pathological proteins Tau64 and 69 are specifically expressed in the somatodendritic domain of the degenerating cortical neurons during Alzheimer's disease. Acta Neuropathol. 80: 111-117.

10 Flament S., Delacourte A. (1990) Tau Marker? Nature 346: 6279.

Ghanbari H., Kozuk T., Miller B., Riesing S. (1990) A sandwich enzyme immunoassay for detecting and measuring Alzheimer's disease-associated proteins in human brain tissue. J. Clin. Laboratory Anal. 4: 189-192.

15

Goedert M., Spillantini M., Jakes R., Rutherford D., Crowther R. (1989) Multiple isoforms of human microtubule-associated protein tau: sequences and localisation in neurofibrillary tangles of Alzheimer's disease. Neuron. 3: 519-526.

20 Goedert M., Cohen E., Jakes R., Cohen P. (1992) p42 Map kinase phosphorylation sites in microtubule-associated protein tau one dephosphorylated by protein phosphatase 2A1: implications for Alzheimer's disease. FEBS Lett. 312: 95-99.

Goedert M., Jakes R., Crowther R., Six J., Lübke U., Vandermeeren M., Cras P., Trojanowski
25 J.Q., Lee V. (1993) The abnormal phosphorylation of tau protein at serine 202 in Alzheimer's disease recapitulates phosphorylation during development. Proc. Natl. Acad. Sci. (USA) 90: 5066-5070.

Greenberg S., Davies P. (1990) A preparation of Alzheimer paired helical filaments that displays
30 distinct tau proteins by polyacrylamide gel electrophoresis. Proc. Natl. Acad. Sci. USA 87: 5827-5831.

28

Harrington C., Mukaetova E., Hills R., Edwards P., Montejo de Garcini E., Novak M., Wischik C. (1991) Measurement of distinct immunochemical presentations of tau protein in Alzheimer's disease. Proc. Natl. Acad. Sci. (USA) 88: 5842-5846.

5

Hasegawa M., Morishima-Kawashima M., Takio K., Suzuki M., Titani K., Ihara Y. (1992) Protein sequence and mass spectrometric analyses of tau in Alzheimer's disease brain. J. Biol. Chem 267: 17047-17054.

10   Himmler A. (1989) Structure of the bovine tau gene: alternatively spliced transcripts. Mol. Cell Biol. 9(4): 1389-96.

Huang Q., He G., Lan Q., Li X., Qian Z. Chen J. Lu Z., Du Z. (1996) Target imaging diagnosis of human brain glioma. Clinical analysis of 40 cases. Nucl. Med. Commun. 17: 311-316.

15

Hulstaert F., Blennow K., Ivanoiu A., Schoonderwaldt H.C., Riemenschneider M., De Deyn P.P., Bancher C., Cras P., Wiltfang J., Mehta P.D., Iqbal K., Pottel H., Vanmechelen E., Vanderstichele H. (1999) Improved discrimination of AD patients using β-amyloid$_{(1-42)}$ and tau levels in CSF. Neurology 52: 1555-1562.

20

Iqbal K., Grundke-Iqbal I., Smith A., George L., Tung Y., Zaidi T. (1989) Identification and localisation of a Tau peptide to paired helical filaments of Alzheimer's disease. Proc. Natl. Acad. Sci. (USA) 86: 5646-5650.

25   Ksiezak-Reding H., Liu W.K., Yen S.H. (1992) Brain Res. 597: 209-219.

Lee V., Balin B., Otvos L., Trojanowski J. (1991) A68: a major subunit of paired helical filaments and derivatized forms of normal tau. Science 251(4994): 675-8.

30   Lewis S., Wang D., Cowan N. (1988) Microtubule-associated protein MAP2 shares a microtubule binding motif with Tau protein. Science 242: 936-939.

Mariani G., Lasku A., Pau A., Villa G., Motta C., Calcagno G., Taddei G.Z., Castellani P., Syrigos K., Dorcaratto A., Epenetos A.A., Zardi L., Viale G.A. (1997) A pilot pharmacokinetic and immunoscintigraphic study with the technetium-99m labelled monoclonal antibody BC-1 directed against oncofetal fibronectin in patients with brain tumours. Cancer 15: 2484-2489.

Mohoney F.I., Barthel D.W. (1965) Functional evaluation: The Barthel Index. Md. State. Med. J. 24: 61-69.

Raichle M.E. (1998) Behind the scenes of functional brain imaging: A historical and physiological perspective. Proc. Natl. Acad. Sci. USA 95: 765-772.

Sandrock D., Verheggen R., Helwig A.T., Munz D.L., Markakis E., Emrich D. (1996) Immunoscintigraphy for the detection of brain abscesses. Nucl. Med. Commun. 17: 311-316.

Scandinavian Stroke Study Group (1985) Multicenter trial of hemodilution in ischemic stroke: Background and study protocol. Stroke 16: 373-403.

Selden S., Pollard T. (1983) Phosphorylation of microtubule-associated proteins regulates their interaction with actin filaments. J. Biol. Chem. 258(11): 7064-71.

Tamada K., Fujinaga S., Watanabe R., Yamashita R., Takeuchi Y., Osano M. (1995) Specific deposition of passively transferred monoclonal antibodies against herpes simplex virus type 1 in rat brain infected with the virus. Microbiol-Immunol. 39: 861-871

Vandermeeren M., Mercken M., Vanmechelen E., Six J., Van de Voorde A., Martin J., Cras P. (1993) Detection of tau proteins in normal and Alzheimer's disease fluid with a sensitive sandwich enzyme linked assay J. Neurochem. 61: 1828-1834.

Van de Voorde A., Vanmechelen E., Vandermeeren M., Dessaint F., Beeckman W., Cras P. (1995) Detection of tau in cerebrospinal fluid. Research Advances in Alzheimer's disease and

related disorders, Ed. Ibqal, Mortimer, Winblad & Wisniewski, John Wiley & Sons Ltd.

Wakabayashi T., Yoshida J., Okada H., Sugita K., Itoh K., Tadokoro M., Ohshima M. (1995)
Radioimaging of human glioma by indium-11 labelled G-22 anti-glioma monoclonal antibody.
Noshuyo-Byori 12: 105-110.

Wilson J.D., Braunwald E., Isselbacher K.J., Petersdorf R.G., Martin J.B., Fauci A.S., Root
R.K. (1991) Harrison's Principles of Internal Medicine, 12th Edition, McGraw-Hill Inc, NY,
USA.

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CLAIMS

1. A method for the early detection and/or quantification of CNS damage in an individual, said CNS damage being caused by space-occupying lesions of the CNS, by invasion or metastasis of the CNS, by organisms, by anoxia or ischemia, by chemical agents, by physical agents or by a combination of these mechanisms, said method comprising the step of determining the level of tau in said individual and comparing it to the level of tau in control healthy individuals.

2. A method for the early in vitro detection and/or quantification of CNS damage in an individual, said CNS damage being caused by space-occupying lesions of the CNS, by invasion or metastasis of the CNS, by organisms, by anoxia or ischemia, by chemical agents, by physical agents or by a combination of these mechanisms, said method comprising the steps of:
      - obtaining a sample from said individual;
      - determining the level of tau in said sample and comparing it to the level of tau in control healthy individuals.

3. A method according to claim 2 in which the sample is taken from the cerebrospinal fluid of the individual.

4. A method according to claim 2 in which the sample is taken from the blood derivatives of the individual.

5. A method according to any of claims 1 to 4 in which the space-occupying lesion of the CNS is a primary brain tumor, benign or malignant, brain metastasis, or a subdural haematoma.

6. A method according to any of claims 1 to 4 in which the invasion or metastasis of the CNS is by leukemia, lymphoma or breast cancer.

7. A method according to any of claims 1 to 4 in which the organisms are bacteria or viruses causing encephalitis or meningitis.

8. A method according to any of claims 1 to 4 in which the anoxia or ischemia is caused by stroke, by cerebral infarction, by cerebral hemorrhage, by thrombosis, by perinatal asphyxia, by Binswanger disease or by vasculitis.

9. A method according to any of claims 1 to 4 in which the chemical agent is.

10. A method according to any of claims 1 to 4 in which the physical agent is a trauma, stroke, intracranial pressure or radiation.

11. A method according to any of claims 1 to 10 in which CNS damage is detected and/or quantified in order to evaluate the effect of a certain treatment on said CNS damage.

12. The use of tau as an aspecific marker for the manufacture of a diagnostic kit for the early detection and/or quantification in an individual of CNS damage caused by space-occupying lesions of the CNS, by invasion or metastasis of the CNS, by organisms, by anoxia or ischemia, by chemical agents, by physical agents, or by a combination of these mechanisms.

13. The use of tau as an aspecific marker according to claim 12 in any method according to claims 1 to 11.

14. A kit for the early diagnosis of CNS damage in an individual, said CNS damage being caused by space-occupying lesions of the CNS, by invasion of the CNS, by organisms, by anoxia or ischemia, by chemical agents, by physical agents, or by a combination of these mechanisms, comprising a tool for the detection of tau.

15. A kit according to claim 14 for use in any method according to claims 1 to 11.

16. A kit according to claims 13 and/or 14 characterised in that said kit comprises:
    - a monoclonal antibody (primary antibody) which forms an immunological complex with an epitope of tau;
    - a secondary antibody

- which can be a monoclonal antibody recognising an epitope of the tau-primary antibody complex, but not recognising the primary antibody alone, or

- which can be a polyclonal antibody recognising an epitope of the tau-primary antibody complex but not recognising the primary antibody alone, with said polyclonal antibody being preferably purified by immunoaffinity chromatography using immobilized tau or immobilized tau-primary antibody complex;

  - a marker either for specific tagging or coupling with said secondary antibody;

  - appropriate buffer solutions for carrying out the immunological reaction between the primary antibody and the test sample, between the secondary antibody and the tau-primary antibody complex and/or between the secondary antibody and the marker;

  - possibly, for standardisation purposes, a purified protein or a synthetic peptide containing one or more tau epitopes.

17. A method to screen or monitor the effect of compounds which prevent or treat CNS damage comprising the step of determining the level of tau and comparing it to the level of tau in a control sample.
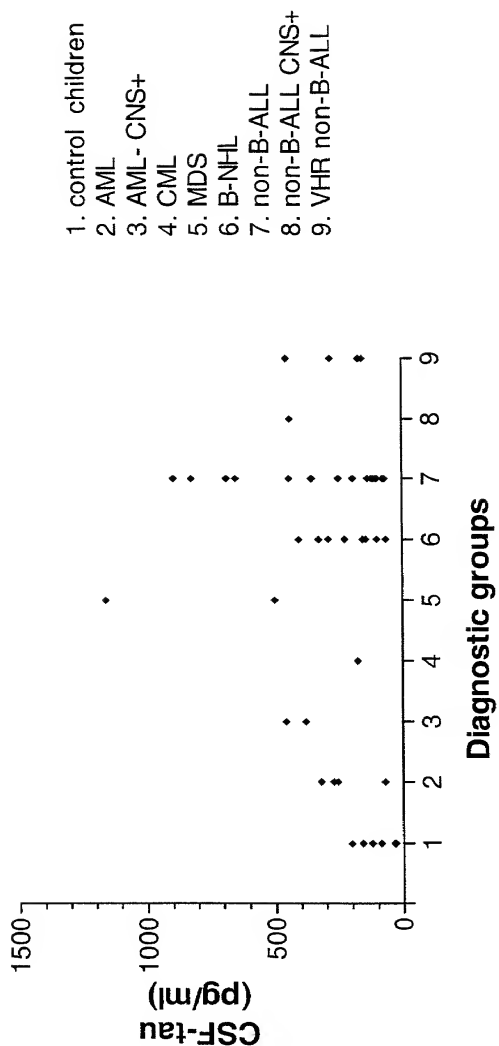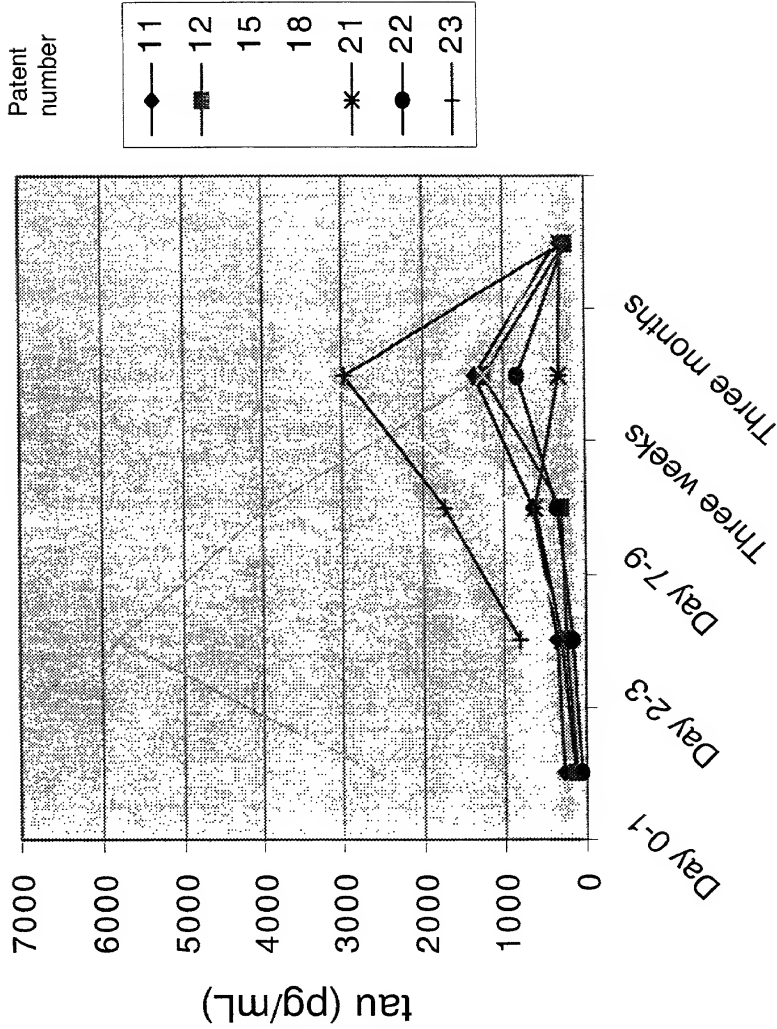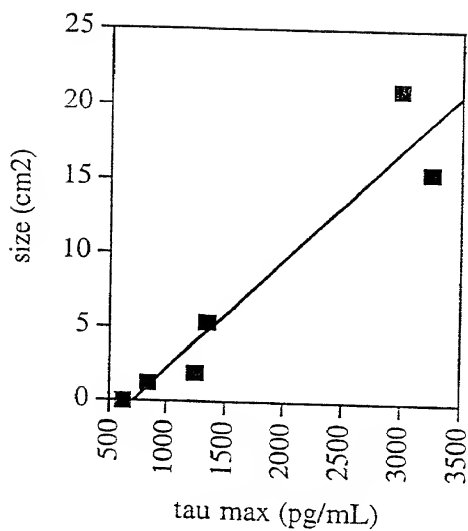
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Figure 1

Figure 2

Figure 3

WE HEREBY DECLARE THAT ALL STATEMENTS MADE OF OUR OWN KNOWLEDGE ARE TRUE AND THAT ALL STATEMENTS MADE ON INFORMATION AND BELIEF ARE BELIEVED TO BE TRUE; AND FURTHER THAT THESE STATEMENTS WERE MADE WITH THE KNOWLEDGE THAT WILLFUL FALSE STATEMENTS AND THE LIKE SO MADE ARE PUNISHABLE BY FINE OR IMPRISONMENT, OR BOTH, UNDER SECTION 1001 OF TITLE 18 OF THE UNITED STATES CODE AND THAT SUCH WILLFUL FALSE STATEMENTS MAY JEOPARDIZE THE VALIDITY OF THE APPLICATION OR ANY PATENT ISSUED THEREON.

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DECLARATION

As below named inventors, we hereby declare that:

Our residence, post office address and citizenship are as stated below next to our names.

The below named inventors are the original, first and joint inventors of the subject matter which is claimed and for which a patent is sought on the invention entitled **TAU AS A MARKER FOR EARLY CNS DAMAGE**, the specification of which was filed as PCT International Application No. **PCT/EP99/06592** on 7 September 1999 and accorded **U.S. Serial Number** _____ .

We hereby state that we have reviewed and understand the contents of the above identified specification, including the claims.

We acknowledge the duty to disclose to the Patent and Trademark Office all information known to us to be material to patentability of the subject matter claimed in this application, as "materiality" is defined in Title 37, Code of Federal Regulations, § 1.56.

We hereby claim foreign priority benefits under Title 35, United States Code, § 119 (a)-(d) of any foreign application(s) for patent listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

PRIOR FOREIGN APPLICATION(S)			Priority Claimed
98870190.0	Europe	08 September 1998	Yes
(Number)	(Country)	(Date Filed)	

We hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, we acknowledge the duty to disclose all information known to me to be material to patentability of the subject matter claimed in this application, as "materiality" is defined in Title 37, Code of Federal Regulations, § 1.56, which become available between the filing date of the prior application and the national or PCT international filing date of this application.

PCT/EP99/06592	7 September 1999
(International Application No.)	(International Filing Date)

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Declaration of Frank Hulstaert, Eugeen VanMechelen, & Hugo Vanderstichele

INNS:034

H: 423401(92P5011.DOC)

<div align="right">**PATENT**</div>

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	§
FRANK HULSTAERT	§
EUGEEN VANMECHELEN	§ Group Art Unit: **Unknown**
HUGO VANDERSTICHELE	§
	§ Examiner: **Unknown**
Serial No.:	§
	§ Atty. Dkt. No.: **11362.0034.PCUS00**
Filed:	§ **INNS:034---**
	§
For: **TAU AS A MARKER FOR EARLY CNS**	§ History: **International Application No.:**
DAMAGE	§ **PCT/EP99/06592**
	§ **Filing Date: 7 September 1999**

<u>ELECTION UNDER 37 C.F.R. §§ 3.71 AND 3.73</u>
<u>AND POWER OF ATTORNEY</u>

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

The undersigned, being Assignee of record of the entire interest in the above-identified application by virtue of an assignment recorded in the United States Patent and Trademark Office as set forth below, hereby elects, under 37 C.F.R. § 3.71, to prosecute the application to the exclusion of the inventors.

The Assignee hereby revokes any previous Powers of Attorney and appoints:

Patricia A. Kammerer, Reg. No. 29,775; M. Todd Rands, Reg. No. 46,249; Matthew L. Madsen, Reg. No. 45,594; M. Suzy Strickland, Reg. No. 45,139; Craig M. Lundell, Reg. No. 30,284; and Janelle D. Waack, Reg. No. 36, 300,;

each an attorney or agent of the firm of HOWREY SIMON ARNOLD & WHITE, LLP, as its attorney or agent for so long as they remain with such firm, with full power of substitution and revocation, to prosecute the application, to make alterations and amendments therein, to transact all business in the Patent and Trademark Office in connection therewith, and to receive any Letters Patent, and for one year after issuance of such Letters Patent to file any request for a certificate of correction that may be deemed appropriate.

Pursuant to 37 C.F.R. § 3.73, the undersigned has reviewed the evidentiary documents, specifically the Assignment to INNOGENETICS N.V., referenced below, and certifies that to the best of my knowledge and belief, title remains in the name of the Assignee.

Please direct all communications as follows:

Patricia A. Kammerer
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ASSIGNEE:
INNOGENETICS N.V.

By: _____
Name: Mr. Philippe Archinard
Title: Chief Executive Officer

Date: February 6th, 2001

ASSIGNMENT:
☐ Concurrently filed ✕
☐ Previously recorded
    Date:
    Reel:
    Frames: